ANGULAR LEAF SPOT OF STRAWBERRY: DISEASE CONTROL STRATEGIES AND ASSOCIATION OF *Pseudomonas syringae* WITH LESIONS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my wife Aimee, for without her love, support, and sacrifice, it would not have been possible; to my parents Gary and Ailene and my sisters Dedra and Leah for their love and support, and to my friend Frank Smith, who also has been there for me from the very beginning; I thank you all.

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
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Angular leaf spot (ALS) of strawberry is a bacterial disease caused by *Xanthomonas fragariae*. Cultivars of strawberry used in Florida vary in their susceptibility to ALS and there are no strawberry cultivars that are immune. Recommendations for management of ALS include using pathogen-free stock, limiting overhead irrigation, and applying chemical treatments. A trial to evaluate various materials for control of ALS was conducted at the Gulf Coast Research and Education Center during the 2005–06 and 2006–07 seasons. During the 2006–07 season, the susceptibility to ALS of various cultivars and breeding selections was evaluated. Spray materials were applied on 7- and 14-day schedules to 'Strawberry Festival' plants and disease severity was evaluated on a scale of 0 to 6. Disease incidence was evaluated at the end of the 2006–07 season. Temperature and rainfall/irrigation data were collected and analyzed for both seasons to assess the effect of environmental conditions on ALS. Treatments that included Actigard at the higher rate, Kocide, Copper Count N, Badge, and Kasumin had significantly lower disease severity than untreated controls. Results from these studies indicate that there are some good alternatives to copper for managing ALS. Cultivars Treasure and Ruby Gem were mildly susceptible to ALS. Cultivars Camino Real, Sweet Charlie, Albion, Festival, Sugar Baby, Carmine, and advanced

selections FL 99-164, Festival #9, and FL 00-51 were moderately susceptible whereas cultivars Winter Dawn and Camarosa, and advanced selections FL 99-117, and FL 01-116 were highly susceptible. Lastly, we report an association between *X. fragariae* and *Pseudomonas* spp. based on laboratory inoculations in which lesions of angular leaf spot were different between plants inoculated with *X. fragariae* only and a mixture of *X. fragariae* and *Pseudomonas* spp.

CHAPTER 1
INTRODUCTION

Florida ranks second in the nation in strawberry production, accounting for about 12 percent of the total U.S. supply of fresh product and generating more than $200 million in sales annually (9). There are several important diseases of strawberry that are costly to control. Most of those diseases are caused by fungal pathogens and are managed through the use of a fungicide program. Angular leaf spot (ALS) is the only economically important bacterial disease of strawberry in the U.S.

Although ALS-like symptoms had been observed in Utah in 1927, ALS was not reported as a bacterial disease caused by *Xanthomonas fragariae* until 1962 (24, 37), when it was observed in Minnesota. No additional reports of the disease occurred until 1966 when ALS was found in Wisconsin, producing severe losses reaching 75–80% of the crop (8). Hildebrand et al. (14) reported that ALS had been observed in California for 10–15 years; however, it was not considered an important disease since its occurrence in the field was sporadic. Hildebrand et al. (14) proposed the name bacterial blight of strawberry rather than angular leaf spot. However, since the symptoms described by Kennedy and King (24) were more frequent than those described by Hildebrand et al. (14), the current name remains angular leaf spot. Finally, in 1971, ALS was reported in Florida (16). ALS had been observed in fields starting in mid-January of 1968 and reports of yield loss and poor plant condition were attributed to ALS. Since evidence of infection was not found in Florida nurseries except in very mild cases during 1969, ALS was not considered to be an important threat. Recent observations by growers in Florida suggest that plantings may now suffer more severe ALS infections and potentially greater losses to the disease.

By 1993, ALS of strawberry was occurring in all parts of the world (49), and it became apparent that the international shipment of infected transplants was responsible for the rapid spread of this disease. At the time of a review by Maas et al. (37), ALS was occurring in North America, as well as Europe, Africa, South America, and Australia; however, efforts to eradicate the disease in Australia were successful (39). In 2001, Janse et al. (18) discovered a similar, but new bacterial disease of strawberry in northern Italy. Although this disease is caused by a bacterium in the genus *Xanthomonas*, symptoms of the two diseases differ. This new disease, bacterial blight, has not been reported in the U.S.

Kennedy and King (24) described the symptoms of ALS as light-green, angular, water-soaked lesions on the under surface of the leaf. The term angular refers to the noncircular appearance of the lesions. When primary infections occur in the interveinal tissue, the vascular system in the leaf limits movement of the pathogen, which limits ALS lesions to the parenchyma tissue. When held to a transmitted light source, the spots are translucent, angular and easily distinguishable from other types of foliar damage during the early stages of infection. If conditions are favorable, these lesions can increase in number and size and eventually coalesce and become necrotic. The advanced symptoms may become more difficult to distinguish from other leaf infections such as common leaf spot, caused by the fungal pathogen *Mycosphaerella fragariae* (24).

Although parenchymatous foliar tissue is targeted primarily, Hildebrand et al. (14) reported that vascular tissue could also be infected resulting in tissue collapse. Symptoms associated with a vascular infection are similar to those of foliar infection in their translucence; but lesions are concentrated in tissue adjacent to veins. Foliar infections are usually randomly distributed on the leaf in contrast to vascular infections. Although not reported as a vascular infection at the time,

examples of both vascular and foliar symptoms can be seen in the first report of ALS on strawberry by Kennedy and King (24). Vascular infections that produce the symptoms associated with lesion concentration in areas adjacent to veins are the result of infection of strawberry crowns by *X. fragariae* (4, 14, 37).

Additionally, blighting of petioles and infection of flowers and runners can occur (11). *X. fragariae* is not pathogenic to the strawberry fruit; however, severe infection of the calyx produces obvious symptoms and the calyx will turn brown making fruit unmarketable (31, 43).

Xanthomonas fragariae is a rod-shaped, Gram-negative bacterium. Typically, the cells are non-motile; however some may have a single polar flagellum (50, 7). *X. fragariae* is aerobic, non-capsulate, and non-spore-forming. Typical colonies are circular, convex, and mucoid. Although they produce xanthomonadin, the colonies are pale yellow and young colonies may appear translucent. Growth on nutrient agar and in nutrient broth is poor. Colony formation may require 3 to 5 days incubation at 20 to 24°C on a suitable medium such as the Wilbrink's medium (26, 48).

X. fragariae is listed as a quarantine pest by the European Plant Protection Organization (7). Often, plants infected with ALS are asymptomatic. Because strawberry is propagated vegetatively and transplants are shipped worldwide, movement of infected transplants often goes undetected and is thought to be the reason for distribution of *X. fragariae* worldwide (37). Thus, good detection techniques are important to avoid movement of the disease.

In the early to mid nineties, efforts to develop rapid detection of ALS with molecular and serological assays resulted in more efficient techniques for confirmation of *X. fragariae*. One technique, Enzyme-Linked Immunosorbent Assay (ELISA), was effective in detecting *X. fragariae* in symptomatic tissue, but, unfortunately, ELISA was ineffective for detection on

asymptomatic tissue or tissue infected with bacterial populations below 10^4 cfu/ml (37, 49). A more effective technique for detection of *X. fragariae* and for detection of epiphytic bacteria is based on the Polymerase Chain Reaction (PCR). With the advent of PCR and its adaptation to plant disease diagnosis, studies began to develop primers for specific detection of *X. fragariae* (13, 45, 47). The molecular and serological detection techniques developed by Rowhani et al. (49), Roberts et al. (47), and Pooler et al. (45) not only improved diagnosis of infected symptomatic material, but hastened the ability to detect the pathogen in asymptomatic transplants as well. The combination of tissue isolation and the new molecular and serological techniques proved successful and diagnosis of ALS was considerably improved (45, 47, 49).

Strawberry production in Florida differs from northern regions in that new transplants are obtained annually from northern or high elevation nurseries to establish strawberry fields. Transplants can be infected and escape detection as leaves may remain asymptomatic until favorable conditions occur. In the study by Roberts et al. (48), nearly 50% of the shipments contained boxes of transplants with ALS symptoms.

To determine the host range of *X. fragariae,* Kennedy and King (24) inoculated 35 different types of plants by infiltration with cell suspensions. None of the thirty-five potential hosts, except strawberry, became infected. Since their initial tests, the only other reported host for *X. fragariae* has been a couple of *Potentilla* species.

Conditions that favor ALS are high humidity, moderate or low temperatures in some cases near zero Celsius, and good plant growth (8, 25). When humidity is high, the underside of the leaf can have a slimy mucous layer of bacterial exudates covering the lesions (8, 36, personal observation). In pathogenicity tests where inoculated plants were incubated under different

moisture conditions, few lesions were observed on plants left on open benches compared to those placed in moisture chambers (8).

Bacterial pathogens are frequently spread by rain as is the case with *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus canker of citrus (3). Another mechanism of bacterial transmission occurs via mechanical transmission by working in the field when plants are wet from rain, or more frequently from the early morning dew. Intuitively, limiting the amount of overhead irrigation during the growing season would be helpful in controlling the spread of ALS in strawberry fields. As early as 1966, Epstein questioned the use of overhead irrigation and pointed out its potential impact on ALS (8). However, in Florida, strawberry is farmed annually and the use of bare-root transplants each season makes overhead irrigation essential for transplant establishment. Florida strawberry field temperatures are high during October, and without adequate overhead irrigation until transplants are established, transplants would dehydrate and die. Additionally, strawberry in Florida is a winter crop and air temperatures can fall below freezing in the evening and early morning hours. Overhead irrigation is the most cost effective method to protect the flowers of the strawberry plants from freezes. Unfortunately, this freeze protection method spreads the pathogen in the field and new symptoms are seen frequently following freeze events (37, 48, personal observation).

Reports of losses associated with ALS are variable. Epstein (8) reported losses from 75–80%; yet in Florida, Roberts et al. (48) reported much lower losses of approximately 8%. No other reports on the effect of ALS on yield have been published since 1997. Thus, more information is needed on the effect of ALS on yield for the currently grown cultivars and on the cost effectiveness of treatment programs. Since the pathogen does not cause fruit rot, and only infects the calyx when disease pressure is high, ALS rarely causes a direct reduction in yield.

However, it may indirectly reduce yield by reducing photosynthetically active leaf area. In a recent study by Mertely et al. (40), leaf and fruit sanitation were compared to the standard fungicide program for management of Botrytis Fruit Rot and leaf sanitation actually reduced yield. A possible explanation suggested by Mertely et al. (40) was that yield loss may be a result of the loss of photosynthetically active tissue and nutrients that could be mobilized by plants brought about by removal of tissue.

Management of plant diseases is usually achieved by employing several techniques. The first method of control is avoidance. Pathogen-free seed or transplants should be used to avoid introducing a pathogen to a field. Sanitation or eradication, the removal of infected tissue or plants, may be useful if applied correctly. Resistance to bacterial pathogens is a useful management tool if available. The use of soil sterilization, anti-bacterial sprays (including inorganic compounds and antibiotics) are other methods for control of plant diseases (1). Some of these techniques may be practical and effective in strawberry production in Florida, but others are not. For example, sanitation has been studied in annual strawberry to manage a different disease and had adverse affects, and antibiotics have not been successful in field application for disease control. The most effective way of preventing ALS is the use of pathogen-free transplants (37, 44). However, disease-free transplants may not be readily available. Copper-based pesticides have been effective for control of bacterial diseases on strawberry and other crops, but phytotoxicity on strawberry plants limit rates and frequencies of application (43). Plant resistance is a useful tool for disease management; however, there are no resistant cultivars of strawberry currently available.

There were four primary objectives of this research. The first objective was to evaluate different spray materials currently available for use in an integrated management program for

control of ALS of strawberry. The second objective was to quantify the effect of ALS and the effect of the treatments for ALS management on marketable yield. The third objective was to determine whether there were differences in susceptibility among commercial cultivars currently grown in Florida or advanced selections from a breeding program. The final objective was to determine if an association of *Pseudomonas* spp. with lesions of angular leaf spot exists.

CHAPTER 2
EVALUATION OF SPRAY MATERIALS FOR CONTROL OF ALS, AND IMPACT OF ENVIORNMENTAL CONDITIONS

Introduction

Angular leaf spot (ALS), caused by *Xanthomonas fragariae*, is a bacterial disease of strawberry (24). The disease was first observed in Minnesota by Kennedy and King in 1959, but was not reported until 1962 (24). ALS is primarily a foliar disease; however the calyx of the fruit can be infected as well. If the calyx is severely infected, the tissue will turn necrotic and fruit is not marketable as fresh fruit (43, 31). Symptoms of ALS in the early stages are easily diagnosed and distinguishable from other foliar infections (24). With ALS, tissue can be infected and asymptomatic making it very difficult to control the spread of infected plant material.

ALS is favored by high humidity and low temperatures similar to those experienced in Florida strawberry fields in the winter months. Temperatures are relatively high during the day, frequently reaching 18 to 22°C, and nights are cool, with air temperatures at ground level dropping to between 0 and 5°C at times. These conditions result in high surface moisture on plants permitting colonization by the pathogen. Studies by Hildebrand et al. (15) and Epstein (8) determined the importance of post-inoculation humidity, and as early as 1966, Epstein (8) questioned the impact that overhead irrigation might have on ALS epidemics.

Losses associated with ALS are not clear. Reports of losses reaching 75 to 80% by Epstein differ greatly from reports in Florida that indicated losses of approximately 8% (8, 48).

Currently, ALS is managed with a variety of techniques. The most effective and efficient technique is the use of pathogen-free planting stock (37, 43). Cultural practices such as limiting overhead irrigation are important in reducing the spread of inoculum in fields. Unfortunately, most growers are dependent on the use of overhead irrigation to protect their crop during freeze events. Currently, the only compounds recommended for control of ALS are copper-based

products. However, there is a danger in using copper-based products since copper is phytotoxic to strawberry plants (48). Thus, new compounds need to be evaluated to determine if better control alternatives exist. Systemic acquired resistance (SAR) inducers, and new antibiotic formulations and biological control agents have shown some promise in managing bacterial diseases and increasing yield in other crops (33, 41, 42). In this study, five types of spray materials were tested including copper-based treatments, SAR inducers, kasugamycin antibiotic treatments, biological control products, and surfactants.

Copper-based spray programs have been proved to be effective to control bacterial spot of tomato, caused by *Xanthomonas campestris*, and citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* (5, 20, 21, 52). In a previous study by Roberts et al. (48), copper hydroxide has shown some suppression of ALS and served as the standard treatment for disease control in this study. Additionally, other copper formulations are available such as basic copper sulfate, copper ammonia complexes, and copper oxychloride. These formulations were evaluated to see whether they were able to reduce disease without causing phytotoxicity.

Products that induce the systemic acquired resistance (SAR) were not available at the time of the study by Roberts et al. (48). Recent studies indicated that the SAR compound acibenzolar-*S*-methyl (ASM) was a good alternative to copper for reducing disease severity in bacterial spot of tomato (41, 35). A group of SAR inducers including ASM were evaluated for efficacy in control or suppression of ALS. Potassium phosphite is a well-known inducer of resistance especially to diseases caused by Oomycetes and has been effective in studies of bacterial spot of peaches, peppers, and tomato (19, 46, 22, 17). The mixture of famoxadone and cymoxanil, although not described as an SAR, is a systemic pesticide that targets protein complex III in the mitochondria thus limiting ATP production and ultimately energy production.

These products penetrate the plant and cannot be washed off by rain. Tanos, the mixture of these chemicals, has been shown to significantly reduce numbers of lesions in bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato* (28, 29).

Antibiotics have not been widely used in the field because of limited residual activity and potential problems with the development of resistance in the pathogen. Recently, Kasumin, a product not yet labeled for use in the U.S. has been studied for control of bacterial diseases of pepper, tomato, citrus, and walnut; all of which are caused by different pathovars of *Xanthomonas campestris* (30, 17). Kasumin significantly reduced disease in all four studies, and is an effective bactericide/fungicide that has been registered and used in other countries for years now. Kasugamycin, the active ingredient in Kasumin, is an aminoglycoside antibiotic from streptomyces, and was included in this study to investigate whether it may also provide control of ALS on strawberries.

Two biological control products were also evaluated—a spore suspension of *Streptomyces lydicus* (Actinovate) and *Bacillus subtilus* (Serenade Max). In separate studies on control of bacterial leaf spot of tomato and pepper, Actinovate and Serenade Max reduced disease compared to the untreated control (17, 33). The bacteria in these products may colonize leaf surfaces of strawberry plants making it more difficult for *X. fragariae* to obtain leaf surface nutrients.

Efforts to improve coverage of pesticides used for control of other economically important strawberry diseases may require a surfactant to be added to the formulation for maximum efficacy. Surfactants alter the properties of water and reduce hydrophobicity to plant surfaces. Unfortunately, the materials used for management of fungal diseases may possibly aid bacterial pathogens in reaching areas with natural openings overcoming the natural physical

barriers of the plant. In a study by Gottwald et al. (10), surfactants were found to enhance bacterial infections on citrus. A surfactant was included in this study to determine if the use of surfactants in strawberry production increases ALS severity.

The goal of this study was to evaluate several new chemical and biological products to determine if any available products minimize or control ALS during the growing season, and by doing so, increase yield. The effects of temperature and rainfall/overhead irrigation on disease severity during the growing season were also analyzed.

Materials and Methods

Field experiments evaluating the use of products for ALS control and the impact of ALS on yield were conducted at the University of Florida Gulf Coast Research and Education Center in Wimauma, Florida during the 2005–06 and 2006–07 growing seasons in fields managed using current conventional strawberry farming practices. On October 6, 2005 and October 16, 2006, bare-root transplants of the cultivar Strawberry Festival from Canada were planted in raised, fumigated beds covered with black plastic mulch. Soil had been fumigated with methyl bromide and chloropicrin at a ratio of 67:33 and at a rate of 397 kg/ha. Beds were 1.2 m apart, center to center, and were 0.7 m wide. Two offset rows of plants were planted on each bed. Plants were spaced 28 cm in the row with 38 cm between the rows. Overhead irrigation was applied for 10 days to establish transplants and then plants were irrigated and fertilized by drip tape. Treatments were arranged in a randomized complete block design in four successive beds. Plots for each treatment contained 12 plants and were 2.9 m long. In this study, 15 and 17 treatments including control groups were compared for the 2005–06 and 2006–07 growing seasons, respectively (Tables 2~1 and 2~2). Transplants were obtained from nurseries known to have ALS to ensure a source of inoculum.

Five types of products were selected for study. These included copper-based products, systemic acquired resistance (SAR) products that induce natural defense mechanisms of the plant, the antibiotic kasugamycin, biological compounds, and a surfactant. In all cases, applications were made with a CO_2 backpack sprayer that delivered 950 L/ha at 275 kPa with a two-nozzle wand. Treatments were applied on a seven-day schedule beginning December 2 and November 22 and continuing through February 22 and February 28 for 2005–06 and 2006–07 seasons, respectively.

Evaluations of disease severity, phytotoxicity, and marketable yield were conducted for both seasons. Disease severity was evaluated three times during each season on a scale from 0 to 6 where 0 = no lesions; 1 = a few lesions on the entire plant; 2 = a few lesions on more than one leaflet with no general necrosis; 3 = several lesions on leaflets with or without concentration of lesions near the veins and necrosis; 4 = numerous lesions present and some partial leaflet blight; 5 = some older leaves killed and others extensively blighted; 6 = all older leaves killed and middle age leaves blighted. For each evaluation, the middle eight plants per plot were rated individually on February 10 and 24, and March 14 for the 2005–06 season, and December 28, January 25, and February 22 for the 2006–07 season. At the end of the 2006–07 growing season, five leaves from six plants in each plot, or a total of 30 leaves or 90 leaflets, were collected arbitrarily and evaluated for disease incidence.

Phytotoxicity evaluations were conducted in all treatments once per season on January 6 and February 28 for the 2005–06 and 2006–07 seasons, respectively, after damage was observed in plots. Phytotoxicity was rated on a scale of: 0 = no phytotoxicity; 1 = light damage; 2 = moderate damage; 3 = severe damage.

Marketable yield was determined by harvesting the fruit twice per week from 20 December, 2005 to 17 March, 2006 and 15 December, 2006 to 16 March, 2007 for a total of 25 and 27 harvests for each season respectively. Fruit was hand picked and graded on the day of harvest. Fruit were considered marketable if there were no visible symptoms of ALS on the calyx or other fruit rot diseases, and fruit weight for each berry was 10 g or greater.

Minimum, maximum, and average temperature and rainfall data were collected for 2005–06 and 2006–07 seasons from the Florida Automated Weather Network (FAWN) and total rainfall and overhead irrigation data were collected from the GCREC weather station for comparison to determine freeze events versus rain events. Increases in disease severity were then compared to dates with heavy precipitation.

Data were subjected to analysis of variance (ANOVA) and the means separated by Least Significant Difference (LSD, $P \leq 0.05$). Statistical analyses of disease severity, disease incidence, phytotoxicity, and yield were performed using software package Statistix 8 (Statistix 8, Tallahassee FL).

Results

2005–06 Chemical Trial

Disease severity

The severity of ALS disease was evaluated on February 10, 24, and March 14, 2006 for the 2005–06 season. An overall disease severity rating for the season was calculated by averaging the disease severity for each treatment for the three evaluation dates. Differences in ALS disease severity were significant for each evaluation period and for the overall season ($P \leq 0.05$). Plants treated with Actigard and Kocide 2000 at the higher rate had significantly lower disease severity than the plants in the untreated control plots for the February 10 evaluation ($P \leq 0.05$). There were no differences between the remainder of the treatments and the untreated control. Plants

treated with Prophyt alternated with Kocide or Kocide + Tanos, Actigard, and Kocide 2000, had significantly lower disease severity compared to plants in the untreated control plots for the February 24 evaluation ($P \leq 0.05$). There were no differences between the remainder of the treatments and untreated control for the February 24 evaluation. Plants treated with Prophyt alternated with Kocide or Kocide + Tanos, and Kocide 2000 had significant differences in disease severity for the March 14 evaluation ($P \leq 0.05$). There were no differences between the remainder of the treatments and untreated control for the March 14 evaluation. Overall, plants treated with Prophyt alternated with copper, Actigard, and Kocide 2000 had significantly less disease than plants in the untreated control plots. Treatments with K-Phite, Prophyt alone or alternated with Actigard or Kasumin, Actinovate, Kasumin, Serenade Max, and Kinetic were not significantly different from the untreated control (Table 2~3).

Phytotoxicity

Phytotoxicity was evaluated on January 6 and there were significant differences between treatments ($P \leq 0.05$). All plots treated with products containing copper had some damage. Plots treated with Actinovate plus Silicone 100, and the untreated control plots had some phytotoxicity; however plots treated with these two products had significantly less phytotoxicity than plots treated with copper-based products. The remaining treatments with no copper component were not phytotoxic (Table 2~3).

Marketable weight

Differences in fruit yield were significant ($P \leq 0.05$) among treatments. Plants treated with Kinetic at 665.5 ml/ha on a 7-day schedule produced 27,516 kg/ha of fresh fruit and plants treated with a mixture of Serenade Max, Biotune, and Kocide on a 7-day schedule produced 20,661 kg/ha fresh fruit for the highest and lowest yields per treatment, respectively. The

untreated control produced 24,291 kg/ha and was not significantly different in yield to any of the other treatments. Therefore, control of ALS did not appear to influence yield (Table 2~3).

Temperature and rainfall

Temperature and rainfall/overhead irrigation data were analyzed from December 1 to March 26 for the 2005–06 season. Two freeze events occurred during this season, on January 8, 2006 and February 14, 2006. Plants received 23.4 mm and 26.9 mm of overhead irrigation on January 8 and February 14, respectively. One major rainfall event occurred during the 2005–06 season on February 3 and 4. Plants received 29.5 mm and 11.2 mm of precipitation, respectively (Figure 2~1A). The freeze event and overhead irrigation coincided with an increase in disease severity observed during the 2005–06 season (Figures 2~1A, 1B).

2006–07 Chemical Trial

Disease severity

The severity of ALS was evaluated on December 28, January 25, and February 22 for the 2006–07 season for all treatments. Disease severity was also evaluated on January 12 and February 9 for the untreated control and a few selected treatments. Differences in ALS severity were significant ($P \leq 0.05$) for each evaluation period and the overall season (Table 2~4). Plants treated with Actigard at the higher rate, Kocide 2000, Copper-Count-N, Badge, and Kasumin + Kocide had significantly lower disease severity than the plants in the untreated control plots for the December 28, January 25, and February 22 evaluations, and for the overall season ($P \leq 0.05$). Plants treated with Kocide 3000 had significantly lower disease severity for the January 25 evaluation ($P \leq 0.05$). Treatments with Cuprofix Ultra 40D, Kinetic, Serenade Max, and Actigard at the lower rate, Kasumin with Captan, and Kasuran were not significantly different from untreated controls in disease severity for the entire season (Table 2~4).

Disease incidence

Differences in disease incidence were significant among treatments ($P \leq 0.05$). All treated plants had significantly lower disease incidence than the plants in the untreated control plots ($P \leq 0.05$). Disease incidence in the untreated control plots was 77% whereas the range of disease incidence in all other plots was between 32% and 58%. Plots treated with Badge, Kocide 3000, Actigard at the lower rate alternated with Kocide, Kasuran, and Copper-Count-N had significantly lower disease incidence with 32%, 37%, 39%, 41%, and 44% disease incidence, respectively. However, those treatments were not significantly different than many of the other treatments (Table 2~4).

Phytotoxicity

Phytotoxicity was evaluated on February 28 and there were significant differences among treatments ($P \leq 0.05$). Plants treated with products containing copper had some damage. Plants treated with Actigard only, Kinetic, and the untreated control had no phytotoxicity symptoms and ratings were not significantly different from each other or from treatments with Cuprofix Ultra, Actigard at the lower rate alternated with Kocide, Kasumin alternated with Kocide, or Kasumin + Captan alternated with Kocide (Table 2~4).

Marketable weight

Differences in fruit yield between treatments were not significant ($P=0.47$). Plants treated with Actigard applied on a 14-day schedule at 26.6 g/ha produced 28,711 kg/ha of fresh fruit and plants treated with Kinetic at 665.5 ml/ha on a 7-day schedule produced 23,794 kg/ha fresh fruit for the highest and lowest numerical yields per treatment, respectively. The untreated control produced 25,375 kg/ha and was not significantly different in yield to any other treatment (Table 2~4).

Temperature and rainfall

Temperature and rainfall/overhead irrigation data were analyzed from December 1 to March 26 for the 2006–07 season. One freeze event occurred during this season on February 17, 2007. Plants received 13.5 mm of overhead irrigation. Nine major rainfall events occurred during the 2006–07 season on the days of December 23 and 25, January 3, 24, 25, and 28, February 2 and 13, and March 16 when plants received 11.2, 30.5, 10.7, 18.0, 15.5, 19.6, 25.1, 16.5, and 12.4 mm of precipitation, respectively (Figure 2~2A). A major increase in disease for the 2006–07 season coincided with the large amount of rainfall and low temperatures in late December. These optimal conditions for ALS were associated with an increase in disease severity from approximately 2.5 to approximately 4.5 in period of one month (Figures 2~2A, 2B).

Discussion

This study demonstrated that suppression of ALS is possible with the use of some products. In the 2005–06 season, disease suppression was achieved mostly with products that contained copper, which was the case in previous studies where copper was used to control bacterial diseases (20, 21). However, Actigard, a non-copper based product that induces systemic acquired resistance, also suppressed ALS, and has controlled bacterial spot of tomato (41). Disease was suppressed most effectively with the higher rate of Kocide 2000, but the disadvantage of this treatment was that it was also phytotoxic. Prophyt treatments when alternated with copper had disease severity indexes lower than untreated controls, but treatments with Prophyt alone did not achieve control. The reduction of disease in the Prophyt/copper treatments was probably a result of the copper, and therefore these treatments were not included in the 2006–07 trial. Actigard on the other hand, contained no copper and suppressed ALS as

effectively as the lower rate of Kocide 2000. The biocontrol agents had no effect on ALS, and Kinetic, a surfactant which was thought might actually increase disease, had no effect.

The 2006–07 trial included some additional copper treatments such as basic copper sulfate and copper oxychloride, but copper hydroxide (Kocide 2000) at the high rate was not evaluated again because of its tendency to be phytotoxic. Additional Actigard treatments, including different rates alternated with copper, were added, and additional antibiotic treatments with higher rates of Kasumin alternated or mixed with copper were added. The additional treatments did not result in any new outcomes. The effective treatments remained copper, Actigard, and Kasumin with copper. Effective copper treatments included copper hydroxide and copper oxychloride + copper hydroxide; however, treatments with copper sulfate were not effective. Treatments with Kasumin were effective; however, these treatments included a copper component and it is likely that efficacy may be due to the copper rather than the antibiotic. Actigard alone again suppressed disease as effectively as the lower rate of copper. At the end of the 2006–07 season, disease incidence was evaluated to determine if disease assessment could be improved. This evaluation proved successful for differentiating all treatments from the untreated control. However, disease incidence did not correlate to disease severity ratings and this assessment would need to be refined for field use throughout the season.

The use of copper for treatment of ALS is of concern to growers because copper is phytotoxic to strawberry plants. Every treatment containing copper for both seasons showed at least some phytotoxicity. Two treatments, that did not even include copper, had trace amounts of phytotoxicity probably from spray drift due to their proximity to treatments receiving copper. Plants that were treated with Kocide at the lower rate had half as much damage as plants treated

with the higher rate. Actigard was the only treatment that provided disease suppression without phytotoxic side effects.

The data on the combined effect of ALS and the management of ALS on yield were inconclusive. In some cases, there were significant differences in marketable yield between treatments, but there were no significant differences in yield between treated and untreated plants for the 2005–06 season. Plants treated with copper at the higher rate had the most phytotoxicity and also had yields similar to plants that were not treated with copper and had no phytotoxicity. Thus, phytotoxicity did not seem to affect yield. The 2006–07 season was similar in that yield differences occurred between a few treatments, but none of the treatments differed from the untreated control. In the 2006–07 season, plants treated with Actigard alone at the lower rate had a significantly lower incidence of disease than untreated plants, but there was no significant difference in marketable yield between the two groups of plants. Contrary to results obtained in tomato (41), it is possible that resistance to ALS in strawberry, whether inherent in the cultivar or artificially induced, could come at a cost to yield. This study also demonstrated the impact that temperature and precipitation or overhead irrigation had on the epidemiology of ALS as previously described (25, 37). Two freeze events occurred during the 2005–06 season during which overhead irrigation was used to protect the crop. The first freeze event occurred on January 8. A month later the first evaluation was conducted, but by this time, ALS had become epidemic. In 2006–07, however, the first evaluation was conducted much earlier in the season, when disease severity was relatively low. Four days prior to the first evaluation there was a major precipitation event which coincided with an extreme drop in temperature. This was not a freeze event, but conditions for development of an ALS epidemic were optimal. About four

weeks after the temperature drop and rain, disease severity had almost doubled. Levels of disease severity were similar to those in the 2005–06 season four weeks after a freeze event.

Despite an increasing number of products labeled for control of ALS, this disease will be difficult to control if conditions are optimal for dissemination of the pathogen. Evaluations of disease incidence rather than disease severity may help to more accurately assess the level of control being achieved since disease incidence is an objective evaluation whereas disease severity is a subjective evaluation. Based on the information that was gathered in this study, Actigard is a good alternative to copper for suppressing ALS in strawberry and providing a level of control similar to copper. There is no phytotoxicity associated with Actigard so plants are not suffering from efforts to manage the disease. Unfortunately, while Actigard appeared to have no negative effect on yield, in this study there was also no positive effect on yield compared to untreated control plants. One possibility for a lack of observable differences in yield may be that plot sizes were too small. Another possibility may be that although differences in disease severity were significant, differences were not enough to reflect differences in yield.

Table 2~1. Treatments and application schedules for control of angular leaf spot in annual strawberry for the 2005–06 season in Florida

Treatment (active ingredient) (rate/ha)	Schedule
Control	
K-Phite (phosphorous acid) (5.8 L)	14-day
Prophyt (potassium phosphite) (5.8 L)	14-day
Prophyt (potassium phosphite) (5.8 L) alt. Actigard (acibenzolar-S-methyl) (53.2 g)	7-day
Prophyt (potassium phosphite) (5.8 L) alt. Kasumin (kasugamycin) (1.2 L)	7-day
Prophyt (potassium phosphite) (5.8 L) alt. Kocide 2000 (copper hydroxide) (1.7 kg)	7-day
Prophyt (potassium phosphite) (5.8 L) alt. [Kocide 2000 (copper hydroxide) (1.7 kg) + Tanos (famoxadone + cymoxanil) (567 g)]	7-day
Actinovate (*Streptomyces lydicus*)(850.5 g) + Silicone 100 (295.8 ml)	7-day
Actigard 50WG (acibenzolar-S-methyl) (53.2 g)	14-day
Kasumin (kasugamycin (1.2 L)	14-day
Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kocide 2000 (copper hydroxide) (1.7 kg)	7-day
Serenade Max (*Bacillus subtilis*) (1.1 kg) + Biotune (sodium lauryl sulfate, sodium dodecylbenzene sulfonate, and polyoxyethylene (20) sorbitan monooleate) (2.9 L)	7-day
Serenade Max (*Bacillus subtilis*) (1.1 kg) + Biotune (sodium lauryl sulfate, sodium dodecylbenzene sulfonate, and polyoxyethylene (20) sorbitan monooleate) (2.9 L) + Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kinetic (organosilicone) (665.5 ml)	7-day

Table 2~2. Treatments and application schedules for control of angular leaf spot in annual strawberry for the 2006–07 season in Florida

Treatment (active ingredient) (rate/ha)	Schedule
Control	
Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kocide 3000 (copper hydroxide) (= GFJ52) (1.0 kg)	7-day
Cuprofix Ultra 40D (copper sulfate) (0.8 kg)	7-day
Copper-Count-N (copper ammonia complex) (2.4 L)	7-day
Badge (copper hydroxide + copper oxychloride) (1.1 L)	7-day
Badge (copper hydroxide + copper oxychloride) (2.1 L)	7-day
Kinetic (organosilicone) (665.5 ml)	7-day
Serenade Max (*Bacillus subtilis*) (1.1 kg) + Biotune (sodium lauryl sulfate, sodium dodecylbenzene sulfonate, and polyoxyethylene (20) sorbitan monooleate) (1.2 L) + Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Actigard 50WG (acibenzolar-S-methyl) (26.6 g)	14-day
Actigard 50WG (acibenzolar-S-methyl) (26.6 g) alt Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Actigard 50WG (acibenzolar-S-methyl) (53.2 g)	14-day
Actigard 50WG (acibenzolar-S-methyl) (53.2 g) alt Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kasumin (kasugamycin) (295.8 ml = 100 ppm) alt. Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kasumin (kasugamycin) (295.8 ml) + Kocide 2000 (copper hydroxide) (0.9 kg) alt Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kasumin (kasugamycin) (5.8 L) + Captan 80WDG (captan) (1.7 kg) alt Kocide 2000 (copper hydroxide) (0.9 kg)	7-day
Kasuran (kasugamycin + copper oxychloride) (2.0 kg = 100 ppm)	7-day

Table 2~3. Severity of angular leaf spot, phytotoxocity, and market weight of annual strawberry for the 2005–06 season in response to the application of various spray materials.

Treatment	DSI [z]1	DSI2	DSI3	DSIAvg	Phytotox[y]	Mkt Wt (kg/ha)
Control	4.94ab[x]	5.44abc	6.03abc	5.47abc	0.50de	24291abcd
K-Phite (5.8 L)	4.38abc	5.32abcd	5.69bcd	5.13bcde	0.00e	25678abc
Prophyt (5.8 L)	5.11ab	5.66ab	6.17ab	5.64ab	0.00e	27054ab
Prophyt (5.8 L) alt. Actigard (53.2 g)	4.56ab	5.16bcde	5.56cde	5.09cde	0.00e	27097ab
Prophyt (5.8 L) alt. Kasumin (1.2 L)	4.41abc	5.47abc	5.88abc	5.25abcd	0.00e	26474ab
Prophyt (5.8 L) alt. Kocide 2000 (1.7 kg)	4.32bc	4.85def	5.32de	4.83def	1.75b	22355cd
Prophyt (5.8 L) alt. [Kocide 2000 (1.7 kg) + Tanos (567 g)]	4.60abc	4.85def	5.07e	4.83def	1.00cd	26528ab
Actinovate (850.5 g) + Silicone 100 (295.8 ml)	5.12ab	5.78a	6.37a	5.76a	0.25e	26671ab
Actigard 50WG (53.2 g)	3.85cd	4.79ef	5.50cde	4.71ef	0.00e	23603bcd
Kasumin (1.2 L)	4.94ab	5.34abcd	5.94abc	5.41abc	0.00e	25516abc
Kocide 2000 (0.9 kg)	3.88ab	4.38fg	5.29de	4.51f	1.25bc	24611abc
Kocide 2000 (1.7 kg)	3.18d	4.03g	4.47f	3.89g	2.50a	21928cd
Serenade Max (1.1 kg) + Biotune (2.9 L)	5.04ab	5.75a	6.25ab	5.68a	0.00e	21993cd
Serenade Max (1.1 kg) + Biotune (2.9 L) + Kocide 2000 (0.9 kg)	4.88ab	5.12cde	5.89abc	5.29abcd	1.00cd	20661d
Kinetic (665.5 ml)	5.19a	5.69a	6.19ab	5.69a	0.00e	27516a

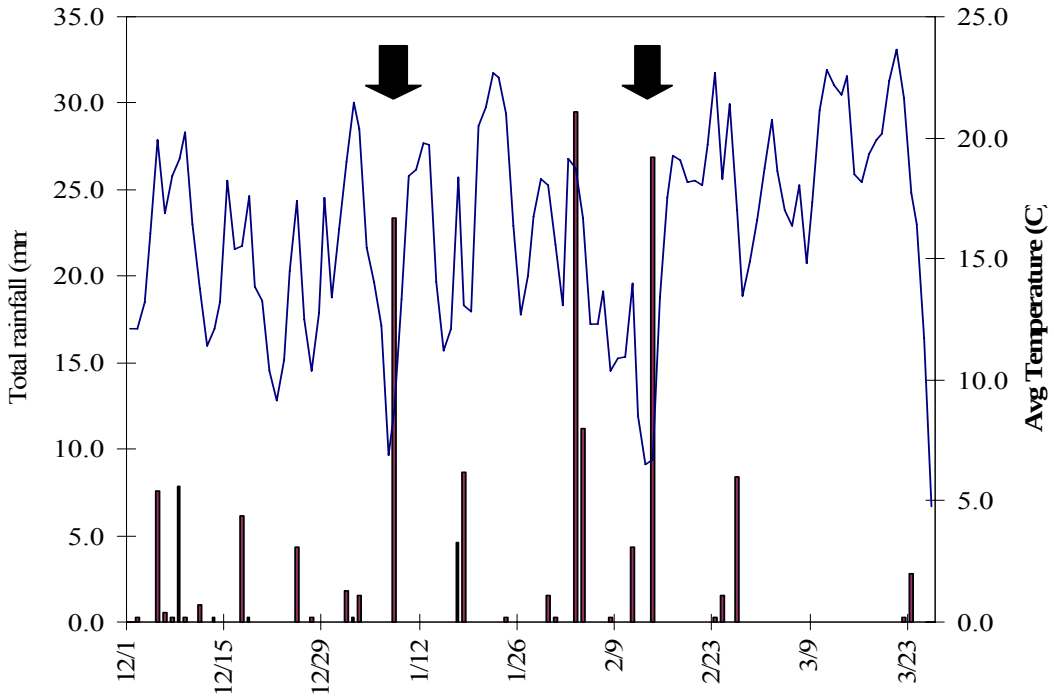
[z] DSI = disease severity index on a scale of 0 = none to 6 = severe. Evaluations were conducted on 2/10, 2/24, and 3/14/2006; [y] Phytotoxicity rated on a scale of 0 = none to 3 = severe; [x] Treatment means within columns followed by the same letter are not significantly different by Fisher's protected LSD ($P \leq 0.05$)

Table 2~4. Severity of angular leaf spot, phytotoxocity, and market weight of annual strawberry for the 2006–07 season in response to the application of various spray materials.

Treatment	DSI [z]1	DSI2	DSI3	DSIAvg	Dis incidence[w]	Phytotox[y]	Mkt Wt (kg/ha)
Control	2.35a[x]	4.28a	4.50a	3.71a	77a	0.00d	25375abc
Kocide 2000 (0.9 kg)	1.00ef	3.16e	3.79f	2.65f	49bcde	1.00bc	28030ab
Kocide 3000 (1.0 kg)	1.91abcd	3.54bcde	4.25abcde	3.23abcde	37efg	1.00bc	25547abc
Cuprofix Ultra 40D (0.8 kg)	1.88abcd	3.78abcde	4.40abc	3.56abcd	45cdef	0.50cd	26016abc
Copper-Count-N (2.4 L)	1.44cdef	3.47bcde	4.03bcdef	2.98cdef	44cdefg	1.25ab	26038abc
Badge (1.1 L)	1.47cdef	3.44cde	4.00cdef	2.97cdef	36fg	1.00bc	27482ab
Badge (2.1 L)	1.44cdef	3.22de	3.88ef	2.85def	32g	1.75a	24860bc
Kinetic (665.5 ml)	2.22ab	4.10ab	4.35abcd	3.56ab	48bcdef	0.00d	23794c
Serenade Max (1.1kg) + Biotune (1.2L) + Kocide 2000 (0.9 kg)	2.22ab	4.00abc	4.54a	3.58ab	56bc	1.25ab	27086abc
Actigard 50WG (26.6 g)	1.97abc	3.85abcd	4.44ab	3.42abc	53bcd	0.00d	28711a
Actigard 50WG (26.6 g) alt Kocide 2000 (0.9 kg)	1.82abcd	3.85abcd	4.38abc	3.35abcd	39efg	0.25d	27456ab
Actigard 50WG (53.2 g)	0.88f	3.28de	3.81f	2.66f	53bcd	0.00d	26595abc
Actigard 50WG (53.2 g) alt Kocide 2000 (0.9 kg)	1.63abcde	3.41cde	4.00cdef	3.0125cdef	45cdef	1.00bc	25331abc
Kasumin (295.8 ml = 100 ppm) alt. Kocide 2000 (0.9 kg)	1.50bcdef	3.69abcde	4.17abcdef	3.12bcdef	47bcdef	0.25d	25461abc
Kasumin (295.8 ml) + Kocide 2000 (0.9 kg) alt Kocide 2000 (0.9 kg)	1.22def	3.28de	3.94def	2.82ef	45cdef	1.00bc	25983abc
Kasumin (5.8 L) + Captan 80WDG (1.7 kg) alt Kocide 2000 (0.9 kg)	1.63abcde	3.85abcd	4.38abc	3.28abcde	58b	0.25d	25780abc
Kasuran (2.0 kg = 100 ppm kasugamycin + Cu)	1.88abcd	3.85abcd	4.28abcde	3.34abcde	41defg	1.50ab	27661ab

[w] = Percentage of disease incidence on a total of 120 randomly collected leaves for each treatment. Evaluation was conducted on 3/31/2007; [x] = Treatment means within columns followed by the same letter are not significantly different by Fisher's protected LSD ($P \leq 0.05$); [y] = Phytotoxicity rated on a scale of 0 = none to 3 = severe; [z] DSI = disease severity index on a scale of 0 = none to 6 = severe. Evaluations were conducted on 12/28/2006, 1/25, and 2/22/2007.

A



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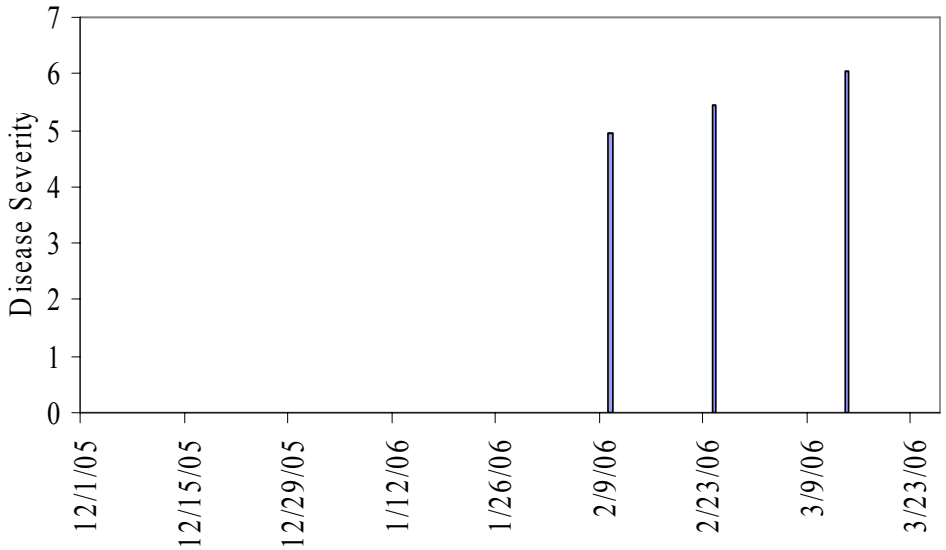
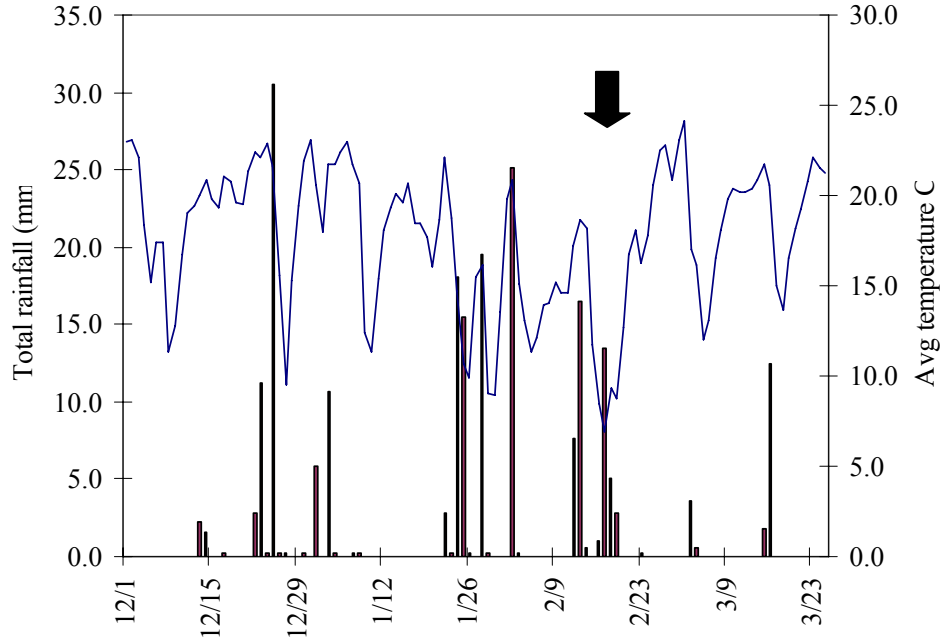


Figure 2~1. Environmental conditions and disease severity for 2005–06. A) Total rainfall/overhead irrigation (mm, vertical bars) and average daily temperature (°C, line) during the 2005–06 annual strawberry season for evaluation of products for control of angular leaf spot. Arrows indicate freeze events when overhead irrigation was used. B) Angular leaf spot disease severity index on leaves of control plots of cultivar Strawberry Festival over time in 2005–06 annual strawberry season.

A



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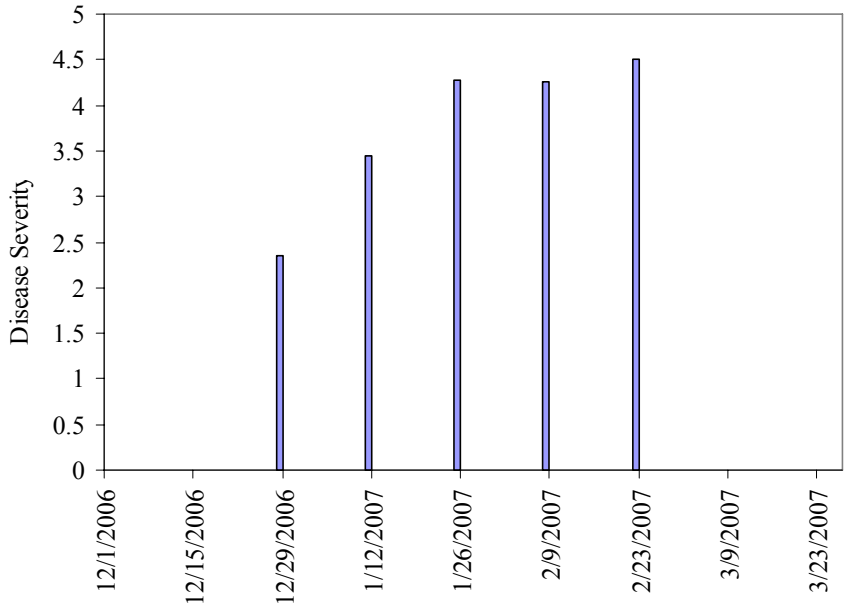

Figure 2~2. Environmental conditions and disease severity for 2006–07. A) Total rainfall/overhead irrigation (mm, vertical bars) and average daily temperature (°C, line) during 2006–07 annual strawberry season for evaluation of products for control of angular leaf spot. Arrow indicates freeze event. B) Angular leaf spot disease severity rating for control plots of cultivar Strawberry Festival over time in 2006–07 annual strawberry season.

CHAPTER 3
RESISTANCE OF FLORIDA STRAWBERRY CULTIVARS TO ANGULAR LEAF SPOT

Introduction

Angular Leaf Spot (ALS) of strawberry, caused by *Xanthomonas fragariae*, is a bacterial disease of strawberry first observed in Minnesota in 1959 and subsequently reported in 1962 by Kennedy and King (24). ALS now occurs worldwide and it is thought that the shipment of infected asymptomatic tissue is responsible for its rapid distribution (37). Symptoms of ALS as described by Kennedy and King (24) are lesions on the abaxial surface of the leaf that are green, angular, and water-soaked. Lesions are translucent and easily distinguishable from healthy tissue. Diagnosis of ALS in the early stage, before lesions become necrotic, is easily done by observing translucent lesions with transmitted light. Once necrosis occurs, diagnosis becomes more difficult as symptoms can be confused with other foliar diseases such as common leaf spot caused by the fungal pathogen *Mycosphaerella fragariae* (24).

ALS is primarily a foliar pathogen of strawberry plants; however infection can occur in the parenchymatous tissue of the calyx. Strawberry fruit itself is not parasitized; however, if infection of the calyx is severe enough, the tissue can become necrotic making fruit unmarketable as fresh fruit (31, 43). Less commonly, *X. fragariae* can infect crowns of strawberry plants resulting in a vascular infection (4, 14, 37). In vascular infections, lesions are concentrated around vascular tissue rather than distributed randomly over leaf surface. At the time of Kennedy and King's first report of ALS in strawberry, *X. fragariae* was not thought to infect vascular tissue; however, their report contains images of leaves with vascular infection as well as parenchymatous tissue infection (24).

ALS is favored by high humidity and low temperatures; conditions common to Florida strawberry fields in the winter months. Temperatures are relatively high in the daytime

frequently reaching 18 to 22 °C and nights are cool, with temperatures dropping to between 0 and 5 °C at times. These conditions result in high surface moisture on plants permitting colonization by the pathogen. Optimal conditions make this disease very difficult to control.

Management of ALS requires an integrated approach. The easiest way to control ALS is to use pathogen-free stock (37). Unfortunately, clean plants are not always available, and plants infected with *X. fragariae* are commonly asymptomatic and escape detection until favorable conditions occur. Limiting overhead irrigation and working in fields after plants are dry is useful for limiting the spread of inoculum in fields, however certain times require overhead irrigation in Florida fields. Strawberry is planted in early to mid-October and mid-day temperatures are high. Overhead irrigation is necessary to establish plants in the field before they receive drip irrigation. Additionally, strawberry is a winter crop in Florida and air temperatures can drop below freezing requiring overhead irrigation to protect the flowers of the plants from freezing.

Chemical application of copper compounds has previously been effective (48), however the use of copper products is limited since copper is phytotoxic to strawberry plants (43, 48). Other spray materials were evaluated in this thesis research and the SAR product acibenzolar-*S*-methyl (ASM) was effective in reducing disease severity compared to untreated control without being phytotoxic to the plants.

Resistant cultivars would be useful for management of ALS; however no resistant cultivars have been developed. Kennedy and King (25) evaluated 64 cultivars with *X. fragariae* to observe reactions to ALS and Hildebrand et al. (15) investigated the factors affecting infection and cultivar reaction for 22 cultivars. All cultivars in those studies were susceptible to ALS, and only two, Sweet Charlie and Chandler, are used for production in Florida. Compared to the other cultivars, Sweet Charlie and Chandler were among the most susceptible in Hildebrand's study

(15). Maas et al. (38) identified two small-fruited genotypes, a native *F. virginiana* and a *F. virginiana* x *F. ×ananassa* hybrid, that are highly resistant to ALS, and a recent study by Lewers et al. (32) has led to understanding the number of genes involved and the heritability of resistance to ALS, but further research with larger populations is needed to develop cultivars with resistance and other desirable traits.

There is speculation from growers that among cultivars grown in Florida the currently most popular cultivar Strawberry Festival is more susceptible than others. However, there are no data available to determine if differences in ALS resistance/susceptibility among cultivars currently grown in Florida exist. The objective of this study was to compare resistance to ALS in cultivars of strawberry currently produced in Florida and four advanced selections from a breeding program, and determine the effect ALS has on yield.

Materials and Methods

Field experiments to evaluate different strawberry cultivars for resistance to ALS were conducted at the University of Florida Gulf Coast Research and Education Center in Wimauma, Florida during the 2006–07 growing season in fields managed using current conventional strawberry farming practices. Between October 12 and October 25, 2006, bare-root transplants of the cultivars Albion, Camarosa, Camino Real, Carmine, Strawberry Festival, , Ruby Gem, Sugar Baby, Sweet Charlie, Treasure, Winter Dawn, and advanced selections Festival #9, FL 99-117, FL 99-164, FL 00-51, and FL 01-116 (Table 3~1) were planted in raised, fumigated beds covered with black plastic mulch. Soil had been fumigated with methyl bromide and chloropicrin at a ratio of 67:33 at 397 kg/ha. Centers of the beds were 1.2 m apart and beds were 0.7 m wide. Two rows of staggered plants were planted in each bed. Plants were spaced 28 cm in the row with 38 cm between the rows. Space between plots for each treatment was 1.1 m. Overhead irrigation was applied for 10 days to establish transplants and then plants were

irrigated and fertilized by drip tape. Treatments were arranged in a randomized complete block design with four replications in successive beds. Plots for each treatment contained 12 plants and were 2.9 m long.

Disease incidence of plants naturally infected was evaluated on April 6, 2007. Based on the results from the products evaluations, disease incidence provided a better separation among treatments than disease severity. Thus, disease incidence was selected for this cultivar evaluation. Five leaves were collected from six plants in each plot, for a total of 30 leaves or 90 leaflets. Marketable yield was determined by harvesting fruit twice per week for each plot. Fruit was hand picked and graded twice per week from 12 December, 2006 to 30 March, 2007 for a total of 32 harvests. Fruit were considered marketable if there were no visible symptoms of ALS on the calyx or other fruit rot diseases, and fruit weight for each berry was 10 g or greater. Data were subjected to analysis of variance (ANOVA) and the means were separated using Fisher's Protected Least Significant Difference (LSD, $P \leq 0.05$). Statistical analyses of disease incidence and yield were performed using software package Statistix 8 (Statistix 8, Tallahassee FL).

Results

Cultivar Evaluation

Differences in cultivar susceptibility were significant ($P \leq 0.05$) with the range of percent disease incidence from 21% to 75%. One cultivar, Treasure, had a significantly lower disease incidence compared to all other cultivars with 21% disease incidence (Table 3~1). The genotypes with the highest disease incidence were advanced selections FL 99-117 and FL 01-116 with 75% and 70% disease incidence, respectively, and cultivars Winter Dawn and Camarosa with 74% and 68% disease incidence, respectively (Table 3~1). Nine cultivars had disease incidences that were not significant from each other, but were significantly different from cultivars with the highest or lowest disease incidence. These included 'Camino Real', FL 99-

164, Festival #9, ‘Sweet Charlie’, ‘Albion’, ‘Strawberry Festival’, ‘Sugar Baby’, ‘Carmine’, and FL 00-51 (Table 3~1).

Marketable Weight

Differences in fruit yield between cultivars were significant ($P \leq 0.05$) with a range of 17,976 kg/ha to 41,112 kg/ha. Cultivars producing the most fruit were Strawberry Festival, Camarosa, Camino Real, and Ruby Gem with 41,112, 39,811, 38,403, and 37,938 kg/ha, respectively, and differences between these four were not significant. ‘Sugar Baby’ and Festival #9 produced the least amount of fruit with 17,976 kg/ha and 19,350 kg/ha, respectively, and differences between these two were not significant. Among treatments that produced slightly more fruit than ‘Sugar Baby’ and Festival #9 were advanced selections FL 01-116, FL 99-117, and FL 00-51 which produced 24,739, 24,790, and 24,988 kg/ha respectively. ‘Albion’, ‘Winter Dawn’, ‘Sweet Charlie’, ‘Treasure’, FL 99-164, and ‘Carmine’ had significantly higher yields than ‘Sugar Baby’, Festival #9, FL 01-116, FL 99-117, and FL 00-51 but also had significantly lower yields than ‘Strawberry Festival’, ‘Camarosa’, ‘Camino Real’, and ‘Ruby Gem’ ($P \leq 0.05$). The incidence of ALS was not related to yield ($P=0.08$). ‘Treasure’, the most resistant cultivar, produced 34,098 kg/ha of fruit which was only slightly higher than, and not significantly different from the most susceptible cultivar, Winter Dawn, which produced 31,346 kg/ha. In comparison, the moderately susceptible cultivar Strawberry Festival produced the highest yield of 41,112 kg/ha. Differences in yield could not be attributed to ALS (Table 3~1).

Discussion

This study demonstrates that all cultivars of strawberry grown in Florida are susceptible to ALS; however, the degree of susceptibility varies greatly. ALS susceptibility among Florida cultivars can differ by as much as 50%, as was observed between ‘Treasure’ and ‘Winter Dawn’. No cultivars of strawberry are immune to ALS and for purposes of this discussion, cultivars are

placed in one of three groups: cultivars that are mildly, moderately, or highly susceptible to ALS. Previous studies have evaluated susceptibility in a number of cultivars, however only two, 'Sweet Charlie' and 'Chandler' are currently used in Florida (25, 15). Hildebrand et al. (15) compared the reactions of 22 cultivars of strawberry to ALS. 'Chandler' and 'Sweet Charlie' were included in that study and compared to the other cultivars in that trial, they were highly susceptible. However, compared to cultivars used in Florida, 'Sweet Charlie' is only moderately susceptible. Although 'Chandler' is not used as much as other current cultivars, and although it was not included in this study, it is likely that 'Chandler' would perform similarly to 'Sweet Charlie' based on the similar susceptibilities reported by Hildebrand et al. (15).

'Strawberry Festival' is currently the most popular cultivar of strawberry used in Florida. One reason for the popularity of 'Strawberry Festival', apart from desirable consumer traits, is its high potential yield. Although popular, growers have speculated that 'Strawberry Festival' is highly susceptible to ALS and those questions have been addressed in this study. 'Strawberry Festival' is a moderately susceptible cultivar compared to the other cultivars grown in Florida, with a disease incidence rating of 58.3%. Despite a moderate disease incidence rating, 'Strawberry Festival' produced the highest total yield of all the cultivars tested, slightly over 7000 kg/ha more than 'Treasure', the cultivar most resistant to ALS in our study.

The results from this study are not conclusive to indicate if ALS significantly impacts yield among cultivars with different levels of susceptibility. Differences in yield and disease incidence were significant between cultivars, however there was no correlation observed between disease incidence and yield. One explanation for the lack of differences in yield among cultivars with different levels of susceptibility to ALS is that other important diseases of strawberry also reduced yield in certain cultivars. 'Treasure' and 'Camarosa' are highly susceptible to

anthracnose fruit rot, caused by *Colletotrichum acutatum*, and 'Sweet Charlie' and 'Camino Real' are highly susceptible to gray mold, caused by *Botrytis cinerea*. These two diseases combined caused losses of 13.1, 8.6, 3.8, and 12.0% of the total number of berries harvested from each of these cultivars, respectively (data not shown). In contrast, 'Winter Dawn' and 'Carmine' lost less than 2.0% of the fruit to anthracnose and gray mold. Thus, in addition to natural differences in yield usually observed among cultivars, variable losses due to other diseases were observed making it difficult to correlate differences in yield to ALS. In addition, ALS is a foliar disease and losses are much more difficult to quantify than losses due to fruit diseases because they are not a direct result of infection. In addition, certain cultivars reach peak blooms at different points during the season. 'Winter Dawn' for example is typically planted early because it produces very quickly and has its peak production before 'Strawberry Festival', which has a more steady production throughout the season and usually peaks in late February/March. ALS was evaluated at the end of the season. It may be of interest to see what levels of disease exist at peak production times for different cultivars. Perhaps an experiment comparing plants infected with ALS and plants free of ALS would accurately evaluate the effect of ALS on yield; however, maintaining plots free of ALS in close proximity to plots infected with ALS have been proven to be very difficult.

Based on these findings, it is clear that ALS affects cultivars differently, but, under the conditions that the plants were exposed to during the 2006-07 season, the differences were not dramatic enough to have a significant impact on yield. 'Strawberry Festival' is a good choice based on marketability and it also produces high yields regardless of moderate levels of disease incidence. Efforts to identify resistance while maintaining desirable consumer traits and high yield are not complete, but recent work focused on obtaining a cultivar with all these traits has

been promising. Of the four advanced selections from a breeding program, one was only mildly susceptible. Advanced selection 99-164, with a disease incidence rating of 48.3%, had a low to moderate susceptibility and had numbers similar to 'Treasure' with respect to yield. Recently, Lewers et al. (32) determined that three to four unlinked loci are involved in conferring ALS resistance. They concluded resistant progeny could be selected; however, studies with large populations would be necessary to produce cultivars that were not only resistant to ALS, but were also good producers of quality fruit. The potential for a resistant cultivar is on the horizon and given time, the combination of a resistant cultivar with high yield and quality fruit is a reasonable possibility

Table 3~1. Disease incidence of angular leaf spot in cultivars of annual strawberry in Florida for 2006–07 season.

Cultivar/Selection	Disease Incidence (%)	Mkt Wt (kg/ha)
Albion	55.8cd[x]	29011ef
Camarosa	68.3abc	39811ab
Camino Real	47.5de	38403abc
Carmine	61.8abcd	35289bcd
Strawberry Festival	58.3bcd	41112a
Festival #9	51.0d	19350g
Ruby Gem	32.5ef	37938abc
Sugar Baby	60.0abcd	17976g
Sweet Charlie	55.8cd	31564de
Treasure	20.8f	34098cd
Winter Dawn	74.3ab	31346de
99-117	75.0a	24790f
99-164	48.3de	34897cd
00-51	64.0abcd	24987f
01-116	70.0abc	24739f

[x] Means within columns followed by the same letter are not significantly different by Fisher's protected LSD ($P \leq 0.05$)

CHAPTER 4
ASSOCIATION OF ANGULAR LEAF SPOT LESIONS AND *Pseudomonas* spp. WITH SYMPTOMATIC TISSUE OF STRAWBERRY.

Introduction

Angular Leaf Spot (ALS), caused by *Xanthomonas fragariae*, has been described by Kennedy and King as dark green angular water soaked lesions on the under side of the leaf (24). Under optimal conditions these lesions can increase in size and number, and in early mornings when surface moisture from dew or rain is abundant, a mass of bacterial exudate appears on the under surface covering the water soaked lesions (24, 37, 8, 36, personal observation). The function of this exudate, whether it may be for survival during unfavorable conditions or plugging vascular tissues, is not known (37). Eventually lesions become necrotic and are more difficult to differentiate from other diseases such as common leaf spot caused by *Mycosphaerella fragariae.*

Recently, detection of *X. fragariae* has improved by using serological and molecular techniques (49, 47, 45), however; isolation of the pathogen is still difficult. Isolation of *X. fragariae* requires 3 to 5 days incubation at 20 to 24° C on a suitable medium such as Wilbrink's medium. The morphology and growth characteristics make direct isolation difficult as growth of the bacterium is slow and faster growing saprophytes mask the growth of *X. fragariae* in culture plates (7, 49). Colonies of *X. fragariae* are minute and translucent rather than yellow at the early stages of growth.

During the 2005–06 and 2006–07 strawberry seasons, numerous tissue isolations were performed to obtain *X. fragariae* strains. Isolations were made under optimal conditions when lesions were oozing and symptoms were easily identifiable as ALS; however, *Pseudomonas* spp. were consistently isolated from the lesions. We report on the association of *Pseudomonas* spp. with angular leaf spot lesions from symptomatic tissue on strawberry.

Materials and Methods

Tissue Isolations

During the 2005–06 and 2006–07 strawberry seasons, leaf samples from 'Strawberry Festival', 'Winter Dawn', and 'Sweet Charlie' were collected from fields at the GCREC, and leaf samples from breeding advanced selections 99-117, 01-116, 97-39, and 99-164 were collected from a farm in Floral City, FL. Tissue isolations were performed by dissecting a small piece of tissue containing one lesion (~2mm x 2mm) that exhibited visual oozing when viewed under the stereoscope and placing the lesion in a 1.5 ml microcentrifuge tube with 200µl to 1 ml of sterile water or 0.01 M magnesium sulfate solution ($MgSO_4 \cdot 7H_2O$). Tissue was macerated in tubes with a microcentrifuge pestle and the suspension was streaked on nutrient agar or Wilbrink's media or both. Plates were incubated at 24 to 28° C for 24 to 72 h. Individual colonies were transferred to culture plates to isolate pure cultures.

Hypersensitive Response Tests

Selected strains of *Pseudomonas* spp. isolated from field samples and a strain of *Xanthomonas fragariae* (ATCC 33239) were tested to determine if they induced a hypersensitive response in tomato and tobacco. The fluorescent pseudomonads and *X. fragariae* strains were grown on nutrient agar for 24 hours and on Wilbrinks medium 48 to 72 hours, respectively. The bacteria were suspended in sterile tap water. Mature tobacco leaves and four week old tomato plants were infiltrated with a water control or bacterial suspensions adjusted to 0.1_{OD} at 590 nm. Plants were examined 16 to 48 hours later for a hypersensitive response.

Fatty Acid Analysis

A single colony of each strain was transferred from a nutrient agar culture to trypticase soy broth agar, and after 24 h of growth at 28ºC approximately 40 mg of cells was collected for analysis. The bacterial fatty acids were derivatized to their methyl esters, separated by gas

chromatography, and identified using the Microbial Identification System software (version 3.6; MIDI, Newark, DE).

Oxidase Test

Cultures identified as fluorescent *Pseudomonas* spp. by fatty acid analysis were tested for indophenol oxidase production. *Pseudomonas* spp. were incubated overnight and tested with oxidase reagent droppers (Becton, Dickinson and Company; Sparks, MD.)

Ice Nucleation

Ice nucleation activity was tested for the fluorescent *Pseudomonas* spp. isolated from field samples and *Pseudomonas* spp. received from tissue isolations from California, North Carolina, and Vermont. Suspensions were prepared from 24 h cultures grown on NA and adjusted to A_{590}=0.1. Aluminum weigh dishes were placed on the surface of the alcohol/ice mixture with the temperature at approximately -10°C and 10 drops of suspension from each isolate were placed in the aluminum dish. The number of drops that froze after 1 minute was recorded for each strain.

Pathogenicity Tests

'Strawberry Festival' and 'Sweet Charlie' plants obtained from a region free of ALS in 6-inch pots were inoculated with suspensions of *X. fragariae*, *Pseudomonas* spp., a mixture of both species, or water control (sterile tap water or $MgSO_4$ $7H_2O$). Suspensions were prepared and diluted to A_{590}=0.1 from 24 h *Pseudomonas* spp. cultures, and 72 h *X. fragariae* cultures. Two groups with two plants per treatment, one set left at room temperature and the other placed in a growth chamber, were inoculated and placed in plastic bags immediately following inoculation. The growth chamber was set to temperature fluctuation of 3°C to 18°C and a 12 hour photoperiod. Plants were observed daily for symptoms after one week post-inoculation.

Results and Discussion

Tissue Isolations

Of the numerous leaf samples collected to isolate the causal agent of ALS of strawberry, none resulted in plates that produced a pure culture of *X. fragariae*. Interestingly, many of the plates contained almost pure cultures of white colonies, and on Wilbrink's media, these same colonies produced a green pigment. It appeared that these cultures consistently isolated from leaves with symptoms of ALS were *Pseudomonas* spp. There were six samples from the GCREC and 11 samples from Floral City (Table 4~1). During this same time, we also received 1, 4, and 10 strains isolated from symptomatic ALS lesions from North Carolina, Vermont, and California, respectively, that appeared to be *Pseudomonas* spp. (Table 4~1).

Characterization of Strains

Isolates 13–15, 27–29, and 31–36 were all identified as *Pseudomonas* spp. by fatty acid analysis (Table 4~1). Those same isolates were evaluated to see if they induced hypersensitive responses (HR) in tomato and the oxidase reaction. All but one of the strains, number 33, failed to induce an HR or induced only a weak HR. Oxidase production was weak in most strains tested. Initially these strains tested slightly oxidase positive but eventually turned negative. Two strains, numbers 33 and 36, were oxidase negative. Ice nucleation activity was tested for several strains. Strains 33 from Floral City, 52 and 54 from Vermont, and strains 70 and 71 from California all exhibited strong ice nucleation activity.

Pathogenicity Test

Pathogenicity tests for all *Pseudomonas* spp. strains were negative (Table 4~1). As a result of isolating almost exclusively *Pseudomonas* spp. from the tissue samples, we began to suspect some type of interaction or association between lesions of ALS caused by *X. fragariae* and these *Pseudomonas* spp.

Bacteria employ a number of strategies to successfully colonize host plants and more specifically, epiphytes are bacteria that can colonize and survive on plant surfaces (2). *Pseudomonas syringae* is a well known pathogen of many crops, but it has also been reported as an epiphyte and can exist as aggregates on surfaces of leaves where scarce nutrients are available (6, 12, 27, 23). For example, some *P. syringae* strains produce indole-3-acetic acid (IAA), which is capable of inducing nutrient leakage on the surface of plants thus making nutrients available for survival (2). Many bacteria produce extracellular polysaccharides which can modify the environment by helping cells adhere to leaf surfaces and preventing desiccation of cells (2). Frequently, a mass of bacterial exudate is associated with ALS lesions during periods of high humidity.

Another mechanism that can assist bacteria in colonization is ice nucleation. During freeze events, ice nucleation active bacteria can injure leaf surfaces increasing frost damage to plants which may provide more openings for pathogenic bacterial ingress (6, 34, 53). Given the fact that some of the *P. syringae* strains associated with strawberry were ina+, it is possible that this served some role in colonization of the leaves. However, no symptoms were observed in pathogenicity tests with only *P. syringae* strains on strawberry.

An association of *P. syringae* and a *Xanthomonas* species has previously been reported in citrus lesions by Stall et al. (51). They reported that *P. syringae* was often associated with citrus canker lesions in Argentina. While *P. syringae* by itself produced symptoms typical of citrus blast, an association of *P. syringae* and *Xanthomonas campestris* pv. *citri* produced symptoms not typical of either citrus blast or citrus canker. Pathogenicity tests on strawberry with *X. fragariae*, *P. syringae*, and a mixture of both organisms had different symptoms as well. Two plants inoculated with *X. fragariae* left at room temperature developed typical ALS lesions

between 6 and 14 days that were described by Kennedy and King (24) in laboratory inoculations; however, no bacterial oozing or tissue necrosis developed with these symptoms as was observed in the field (Figure 4~1A). Additionally, the level of humidity had an effect on the expression of these early symptoms. When removed from high humidity, expression of ALS symptoms in plants inoculated with *X. fragariae* was reduced. Plants inoculated only with *P. syringae* did not develop symptoms, but the combination of the two organisms produced symptoms in two plants in the growth chamber that were identical to those seen in the field during the season (Figure 4~1B). After three weeks, lesions on strawberry plants inoculated with a mixture of *X. fragariae* and *Pseudomonas* spp. developed oozing lesions that became necrotic at the site of the lesion. After lesions began oozing, both plants remained in bags but were transfered to room temperature where development of symptoms progressed rapidly.

In order to determine if the *Pseudomonas* strains were colonizing ALS lesions in strawberry, isolations of lesions at various stages of infection were performed and populations of *Pseudomonas* strains were enumerated. Isolations were made from a leaf with no symptoms, early lesions (no oozing), advanced lesions (oozing, no necrosis) and necrotic tissue. Populations of *Pseudomonas* were not different between asymptomatic tissue and early lesions; however, populations increased by three orders of magnitude between early lesions and advanced lesions. Populations on necrotic tissue were too numerous to count (data not shown).

This preliminary study indicates that an association between ALS lesions and *Pseudomonas* strains exists based on the observations that the *Pseudomonas* spp. tested exacerbated the symptoms caused by *X. fragariae*. More data is necessary to statistically compare populations of *Pseudomonas* spp. associated with ALS lesions. Because strawberry was not an apparent host to the *Pseudomonas* strains isolated, and if the *Pseudomonas* spp. are

responsible for the exacerbation of the symptoms, there may be need for a new management strategy for ALS in strawberry.

Table 4~1. Characterization of *Pseudomonas* strains associated with lesions of angular leaf spot on strawberry during 2005-06 and 2006-07 season.

Strain Designation	Location	Colony Color	Fatty Acid	HR	Oxidase	Ice Nucleation	Path Test
12	GCREC, FL	White	ND	Neg	ND	ND	Neg
13	GCREC, FL	White	*Pseudomonas syringae* pv. tomato	Weak	Weak +	ND	Neg
14	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	Neg
15	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Weak	Weak +	ND	Neg
16	Floral City, FL	White	ND	ND	ND	ND	Neg
23	GCREC, FL	White	ND	ND	ND	ND	ND
24	GCREC, FL	White	ND	ND	ND	ND	ND
25	GCREC, FL	White	ND	ND	ND	ND	ND
27	GCREC, FL	White	*Pseudomonas putida* biotype B	Neg	Pos	ND	ND
28	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	ND
29	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	ND
31	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	ND
32	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	ND
33	Floral City, FL	White	*Pseudomonas syringae* pv. *atrofaciens*	Pos	Neg	10/10	Neg
34	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Weak	Weak +	1/10	Neg
35	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Weak +	ND	ND
36	Floral City, FL	White	*Pseudomonas syringae* pv. tomato	Neg	Neg	ND	ND
50	North Carolina	White	ND	ND	ND	2/10	ND
51	Vermont	White	ND	ND	ND	10/10	ND
52	Vermont	White	ND	ND	ND	5/10	ND
53	Vermont	White	ND	ND	ND	10/10	ND
54	Vermont	White	ND	ND	ND	ND	ND
60	California	White	ND	ND	ND	ND	ND
61	California	White	ND	ND	ND	ND	ND
62	California	White	ND	ND	ND	ND	ND
70	California	White	ND	ND	ND	10/10	Neg

71	California	White	ND	ND	ND	10/10	Neg
72	California	White	ND	ND	ND	0/10	ND
73	California	White	ND	ND	ND	0/10	ND
74	California	White	ND	ND	ND	2/10	ND
75	California	White	ND	ND	ND	0/10	ND
79	California	White	ND	ND	ND	1/10	ND

ND = Not determined

A

B

Figure 4~1. Angular leaf spot symptoms on strawberry. A) Angular leaf spot symptoms on strawberry inoculated with *X. fragariae* only. B) Bacterial oozing and slight necrosis from ALS lesions on strawberry inoculated with *X. fragariae* and *Pseudomonas* spp.

CHAPTER 5
SUMMARY

Overall, the goal of this research was to develop more effective management practices for angular leaf spot of strawberry. To accomplish that goal field studies comparing different chemical and biological products were evaluated, cultivar resistance for all cultivars of plants used in Florida was evaluated, differences in yield between treatments and untreated controls were compared, the impact of environmental conditions on control of angular leaf spot was analyzed, and finally a preliminary study on the association of *Pseudomonas* spp. and lesions of angular leaf spot was performed. From these studies we have learned that Actigard is a suitable alternative to copper-based products for suppressing angular leaf spot without inducing the negative side effect of phytotoxicity; however, we were not able to conclusively determine if suppression of angular leaf spot or minimizing the damage from phytotoxicity could increase yield. This study also produced the first data comparing resistance to angular leaf spot for all cultivars grown in Florida. Similarly, we learned that differences occur in cultivar resistance and this resistance may be useful as part of a management strategy; however, the effect of angular leaf spot on yield remains a question. The impact of environmental conditions was analyzed and we learned that if optimal conditions occur in the field; management of this disease will be difficult regardless of methods. Finally, by processing a number of symptomatic tissue samples and performing pathogenicity tests on the strains resulting from isolations, we observed that there was an association between *X. fragariae* and *Pseudomonas* spp. based on laboratory inoculations in which symptoms differed between plants inoculated with *X. fragariae* and *Pseudomonas* spp. More work is required to better understand this interaction. Further studies to understand this association may lead to new more effective management of angular leaf spot.

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BIOGRAPHICAL SKETCH

Gary Todd Cooper was born in Columbus, Ohio to Gary and Ailene Cooper. He and his family moved to southwest Florida and he graduated from Port Charlotte High School in 1987. After high school graduation, he worked in his family's business until 1991. He entered the automotive industry in 1991 and worked as an automatic transmission repair technician. While working as a repair technician, he began his undergraduate degree attending night classes and eventually transitioned into a career in science by accepting a position studying bioaerosols at Battelle Memorial Institute in Columbus, Ohio. He received his Bachelor of Science in life science from Otterbein College after completing eight years of part-time school. Immediately following graduation, he accepted an offer to pursue a Master of Science in plant pathology at the University of Florida. He has been married since October 12, 2002 to Aimee, who is also a graduate of Otterbein College. He is looking forward to pursuing his Ph.D. at the University of Florida in the fall of 2007.